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A CHEMOTAXONOMIC STUDY OF TWELVE SPECIES OF THE FAMILY LORANTHACEAE

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Abstract

The Loranthaceae is a taxonomically difficult family and the generic and specific limits and relationships of several members are in dispute. In an attempt to gain a clearer understanding of the relationships between the taxa, a chemotaxonomic study was carried out, an approach that has recently been used in a number of other families. Initially, a method of extraction and separation of phenolic compounds by paper chromatography is determined that is suitable for use with *Viscum rotundifolium* Linn. f. Using this technique, the phenolic composition of twelve species of the Loranthaceae is examined and compared, thus showing taxonomic relationships between these species with respect to this class of compounds. Suggestions are made as to the possible significance of the use of these substances in studies of the family.

Uittreksel

'N CHEMOTAKSONOMIESE STUDIE VAN TWAALF SPESIES IN DIE FAMILIE LORANTHACEAE

Die Loranthaceae is taksonomies 'n moeilike familie. Die onderskeid tussen genera en spesies is in 'n aantal gevalle onduidelik. In 'n poging om meer duidelikheid te verkry, is 'n chemotaksonomiese studie van die groep onderneem. 'n Tegniek vir die ekstraksie en isolasie van fenoliese verbindings van *Viscum rotundifolium* Linn. f. is eers ontwikkel. Dié metode is toe gebruik om die fenoliese samestellings van twaalf spesies te ondersoek. Taksonomiese verwantskappe ten opsigte van hierdie tipe verbinding is gevind. Voorstelle met betrekking tot die moontlike betekenis van die gebruik van fenole in studies van die familie word gemaak.

Introduction

The Loranthaceae is largely a tropical family but also extends into temperate zones of both hemispheres. All the species are parasitic or semi-parasitic woody plants, commonly called mistletoes, which grow on the stems, branches and roots of many trees and shrubs (Fig. 1). There are about 1 300 species in the Loranthaceae, which have been variously grouped into between 12 and 125 genera by different authors (Gill & Hawksworth, 1961).

More than fifty species have been recorded in South Africa of the two genera *Viscum* and *Loranthus*. Species of *Loranthus* can be recognised by their often very attractive and showy flowers which may be as much as 6 cm long and leaves which often resemble those of the host, making them inconspicuous (Fig. 2). The flowers of *Viscum*, on the other hand, are inconspicuous and the leaves in several species are reduced, often to scale-like outgrowths, their function being replaced by flattened stems or cladodes. (Fig. 3).

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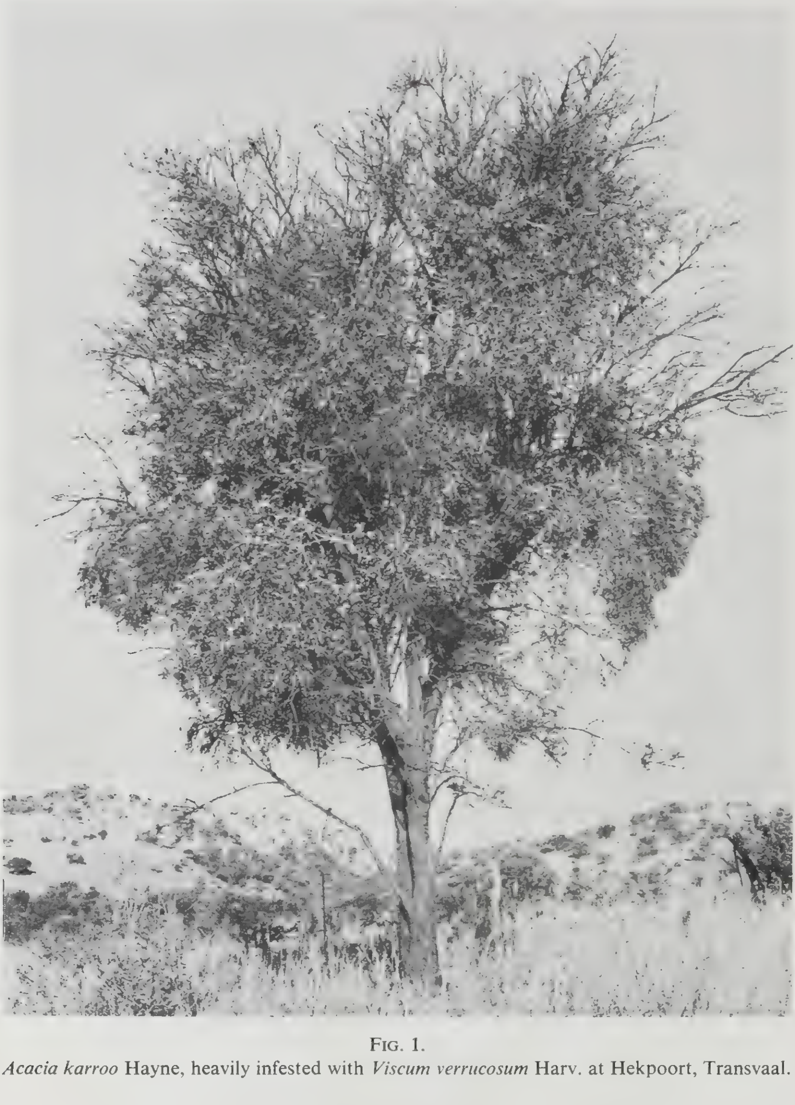


FIG. 1.
Acacia karroo Hayne, heavily infested with *Viscum verrucosum* Harv. at Hekpoort, Transvaal.

On the basis of these floral characters and on the presence or absence of a subfloral bract or calyculus, De Candolle (1830) divided the family into two fairly natural subfamilies, the Loranthoideae, in which the calyculus is always present below the perianth, and the Viscoideae, which lack the calyculus. Another feature which divides these subfamilies is the position of the vascular tissues of the fruit; internal to the viscin layer in the Loranthoideae and external in the Viscoideae (Gill & Hawksworth, 1961). Hegnauer (1963) claimed that the two subfamilies are clearly distinguishable by the production of distinct chemical compounds although their exact nature was not specified.

FIG. 2.
Loranthus rubromarginatus Engl., growing on *Protea caffra* Meisn. at Kenmare, Krugersdorp, Transvaal.

Much taxonomic controversy centres about the genus *Loranthus*, which had approximately 200 species assigned to it by 1830 (Gill & Hawksworth, 1961). The revisions of Danser (1929, 1931, 1933) are widely used in the classification of the Loranthoideae. In the last decade various new schemes have been put forward, but these have been largely restricted to species of particular

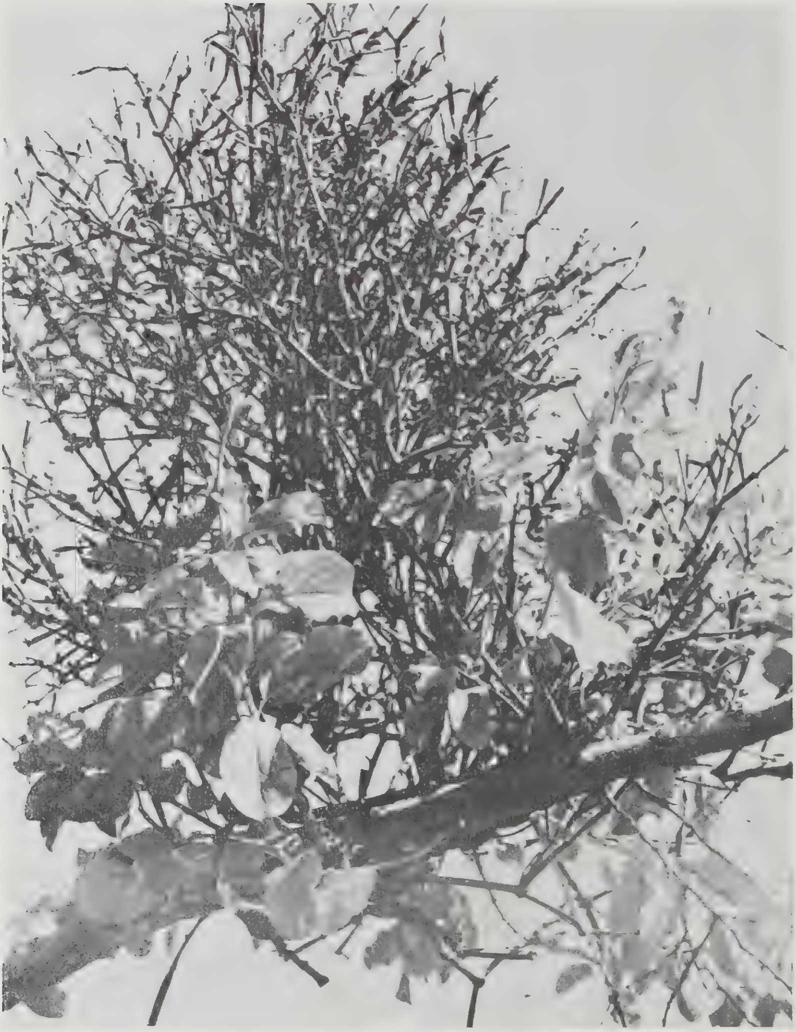


FIG. 3.

Viscum combreticola Engl., a species with no leaves, their function being assumed by the flattened stems or cladodes, seen growing on *Combretum zeyheri* Sond. at Hartebeestpoort Dam, Brits dist., Transvaal.

areas such as South West Africa, (Balle, 1968), and so embrace only relatively few members of the family.

Balle (1956) made a study of the African species of *Loranthus* and these changes will probably be accepted when a thorough study of the South African representatives has been made. As there is still some doubt as to the status of the species of the genus the older taxonomy is adhered to in this study.

A large number of diagnostic features that have been used in the taxonomy of the family have been reviewed by Barlow (1964) who showed how studies of embryology, palynology and chromosome numbers have revealed many differences between the two subfamilies. Consequently a number of authors (Dixit, 1962; Barlow, 1964; Kuijt, 1968; and Wiens and Barlow, 1971) recognise separate families for the Loranthaceae.

In recent years, due largely to recent advances in techniques such as chromatography and electrophoresis, much information concerning the systematic distribution of certain chemical constituents in plant materials has become available. Many of these substances appear to be stable and unambiguous and it is therefore possible to use them as taxonomic characters to aid in determining the natural relationships between taxa.

Since available information indicated that phenolic compounds separated by chromatographic methods could provide valuable taxonomic information, a study of these substances in the Loranthaceae was planned. These compounds have not been as extensively investigated as other substances, such as essential oils and alkaloids (Alston and Turner, 1963).

Chemotaxonomic studies using phenolic compounds have been carried out on a number of genera, including *Baptisia* (Alston and Turner, 1959, 1963), and *Lotus* (Harney and Grant, 1964) among others. They have been found to be more reliable indicators of species relationships in *Baptisia* than are alkaloids or amino acids (Brehm and Alston, 1964).

Phenolics are widely distributed in various parts of the plant body such as leaves, flowers and seeds. Some, such as anthocyanins, can be regarded as commonly occurring. Bate-Smith (1958) found that phenolics in vegetative tissues are of considerably more systematic value than those present in flowers, for the latter are genetically highly variable and, moreover, their chemical composition is frequently not closely correlated with the systematic position of the plants. On the other hand, a large number of leaf constituents are relatively constant and many are diagnostic for the species (Alston and Turner, 1963).

The phenolic composition of some mistletoes has been studied in recent years. Attention has been focused on chemical substances of medicinal significance, for example, some proteins from *Viscum album* are found to inhibit tumour growth (Vester *et al*, 1968). Samuelsson (1966) has been screening a large number of species and genera of the Loranthaceae for toxic proteins. However, the first comprehensive chemotaxonomic study of the mistletoes reported in the literature is that of Hawksworth and Wiens (1972) on the genus of dwarf mistletoes, *Arceuthobium*. A more detailed review of the information on the phenolics of mistletoes is given in their publication. Hawksworth and Wiens (1972) carried out a very detailed taxonomic revision of *Arceuthobium* which included the use of 3 anthocyanins and 17 other phenolics (flavones, flavonols, and cinamic acid ester derivatives) in a taximetric study.

The purpose of the present study on the Loranthaceae was firstly to determine a suitable chromatographic technique for separating different phenolic compounds of the species. The technique was then used to examine the phenolic substances of twelve species to ascertain whether or not such chemical information is likely to be of value in the classification of the family.

MATERIALS

A single species, *Viscum rotundifolium* Linn. f., was chosen for a preliminary analysis to determine a satisfactory chromatographic technique. This species is locally abundant, growing on *Ziziphus mucronata* Willd. Leaves of all ages and stems of two different stages were selected for study.

The comparative chemotaxonomy was made using six species of *Viscum* and six species of *Loranthus* which are readily available in South Africa (Table

TABLE 1.

The species, their hosts and localities used in this study.

Species	Hosts	Locality
Viscum rotundifolium Linn. f.	*Ziziphus mucronata* Willd.	Witpoortjie, Roodepoort dist.
Loranthus dregei Eckl. & Zeyh.	*Acacia mellifera* (Vahl.) Benth.	Rooibokkraal, Waterberg dist.
L. minor Sprague	*Acacia tortilis* (Forsk.) Hayne	Rooibokkraal, Waterberg dist.
L. oleaefolius Cham & Schlecht	*Rhus engleris* Britt.	Pienaars River, Warmbaths dist.
L. rubromarginatus Engl.	*Protea caffra* Meisn.	Witpoortjie, Krugersdorp dist.
L. zeyheri Harv.	*Acacia caffra* Willd.	Witpoortjie, Krugersdorp dist.
L. species	*Rhus lancea* L.f.	Koegas, Orange River, Kuruman dist.
Viscum capense L.f.	*Rhus undulata* var. *burchellii* (Sond.) Schonl.	Sevenfountains, Albany dist.
V. combreticola Engl.	*Combretum molle* R.Br.	Hartebeestpoort Dam, Brits dist.
V. obovatum Harv.	*Vitex pooara* Corb.	Nylstroom dist.
V. obscurum Thunb.	*Scutia myrtino* (Burm. f.) Kurz.	Kasouga, Bathurst dist.
V. rotundifolium Linn. f.	*Boscia albitrunca* (Burch) Gilg and Benedict	Pienaars River, Warmbaths dist.
	Ziziphus mucronata Willd.	Pienaars River, Warmbaths dist.
V. verrucosum Harv.	*Acacia tortilis* (Forsk.) Hayne	Pienaars River, Warmbaths dist.

1). The range of hosts and distribution of the species were determined from herbarium records and recorded fully in Tilney (1970). Both leaves and stems were used where appropriate. *Viscum rotundifolium* was analysed from two different hosts. Voucher specimens are deposited in the Moss herbarium, University of the Witwatersrand.

In a study of this nature on parasitic plants there were two problems that had to be alleviated by the selection of suitable material and choice of technique:

1. A number of *Viscum* species lack leaves or possess very reduced leaves. In order to overcome this difficulty, *Viscum rotundifolium*, which is a leafy species, was investigated with respect to the phenolic compounds of stems and leaves, to see if there is a correlation between the chemical constituents of the two organs. If this is the case, it may be inferred that the modified stems or cladodes of the leafless species are likely to be comparable in their phenolic composition to the leaves of the leafy forms. In a similar situation Bate-Smith (1956) regarded the phyllodes of *Acacia* as "leaves" in a study of their phenolic composition.
2. There is also the possibility in parasitic plants that substances produced in the host plant may accumulate in the parasite. Thus the phenolic composition of the mistletoe may be modified by virtue of the nature of its host. To minimise the possibility of host-parasite translocation affecting the results in the preliminary analysis, leaves and stems were taken from a single specimen. In the comparative taxonomy study, one mistletoe species was selected from two different host species to investigate this effect.

METHODS

Leaves and stems of *Viscum rotundifolium* were ground into a powder and phenolics were extracted and separated according to fifteen different techniques, many being different combinations of extraction methods with different solvents. (Brehm and Ownbey, 1965; Graziano *et al*, 1967; Tilney, 1970). In each case, 0,05 ml of the extract was applied to the chromatogram paper and irrigation was carried out in one-dimension.

The dry chromatograms were examined under ordinary light, and ultraviolet light, and after spraying with a concentrated solution of sodium carbonate. The colours and positions of the component spots obtained with the different techniques were marked (Tilney, 1970).

From this preliminary study, the technique which gave a large number of spots and yet showed close affinity between the spots obtained with leaves and stems, was chosen for the detailed taxonomic study. It was assumed that the technique most successful for *Viscum rotundifolium* would be the most suitable one for the other species.

For each of the twelve species, leaf, cladode or stem material was selected, ground and extracted in acidified methanol. This was left overnight in a dark cupboard and then filtered with Whatman number 3 paper. From the extract obtained, 0,05 ml was applied to Whatman number 3 chromatographic paper and irrigated with n-butanol: acetic acid: water in the ratio 4:1:5 (V/V). Development time was 24 hours. Duplicate extractions thus were performed with each species to ensure constancy of results. The chromatograms were all run at the same time so as to subject them all to exactly the same conditions.

The chromatograms were examined in visible light, under ultra-violet light, after exposure to the fumes from a solution of concentrated aqueous ammonia, and in visible light after being sprayed with azolated benzidine. The latter reagent was found to be superior to sodium carbonate as it gives a wider range of colour reactions with phenolics, so that a greater number of compounds could be distinguished.

The positions and colours of the spots obtained for each species extract were marked on the chromatograms. The spots were numbered and the presence or absence of a spot recorded for each species. R_f values for each spot were calculated and compared with known compounds.

In order to express quantitatively the similarities between all pairs of species, the Paired Affinity (P.A.) Indices were calculated (Alston and Turner, 1963).

$$\text{Paired Affinity} = \frac{\text{Spots in common for species A and B}}{\text{Total spots in A and B}} \times 100$$

where A and B represent all possible pairings between the twelve species.

Following the method of Ellison, Alston and Turner (1962) the P.A. Indices were used to construct polygonal graphs for each species. P.A. Indices are expressed from 0 (at the centre of the circle) to 100 (at the periphery of the circle) along the appropriate radii. Each radius represents a different species compared with the species for which a polygonal graph is being constructed. Since there is always one self comparison one value for each graph will always be 100. The advantage of polygonal graphs is that they enable the pattern of biochemical affinity of the various species with that of all others under study to be visualized quickly and with ease. The greater the similarity in shape shown by the polygonal graphs of any two species the more closely they are likely to be related.

The presence of unique substances within a taxon being investigated is potentially of systematic value. The percentage of unique or distinctive compounds within a species has been termed the isolation value (I.V.) by Ellison, Alston, and Turner (1962). The I.V. can be calculated with respect to the grand total of all different compounds for all species investigated (I.V.n.).

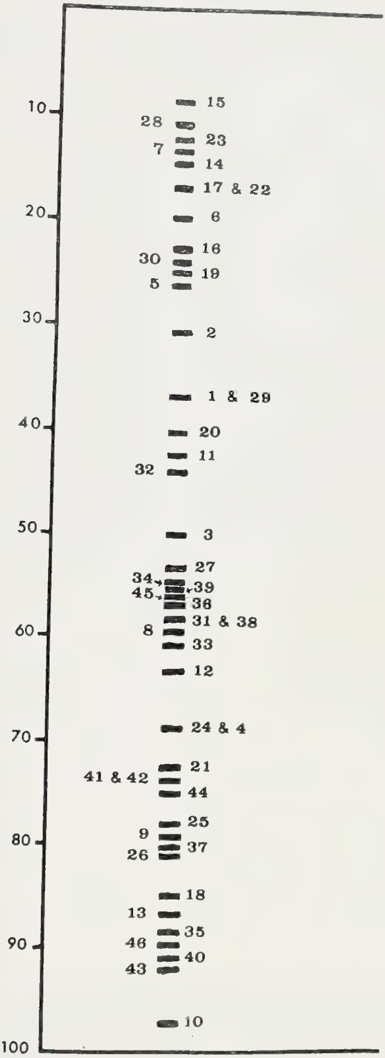


Fig. 4.

Master chromatograph showing the position of each of the thirty-seven compounds and nine standards. The numbers refer to the spot numbers in Table 2.

TABLE 2.

The average Rf values and colour reactions obtained for individual spots and known compounds.

Spot No.	*Av. R_f in 4:1:5 (v/v): Butanol: Acetic acid: water*	*Colours observed**				
		Visible	*Ultra violet*	*NH_3+ ultra-violet*	*NH_3*	*Benzi-dine*
1	37		B	B		P
2	31		B	B		B
3	51		P	Y	Y	Pi
4	69		B	B	Y	Pi
5	27		B	B		P
6	20		B	B		P
7	14	YBr	YBr	YBr		P
8	60		B	B		PBr
9	80		B	B	Y	WP
10	98	G	O	O	G	G
11	43		B	B	Y	
12	64					O
13	87		P	V		O
14	16	OBr	V	B		RBr
15	9	OBr		YBr		
16	23		Pi	PBr		Pi
17	17	RBr	O	O	M	Br
18	86					L
19	26	C	O	Br	M	BrO
20	41		B	V		OBr
21	73	O	P	Y	Y	Pi
22	17		Y	Y		Y
23	13		P	V		
24	69	Y	P	B	Y	RP
25	79		P	Y	Y	Pi
26	82		P	Br	Y	Pi
27	54		V	V		O
28	12	YO	Y	Y		Br
29	37	RO	Y	Br		O
30	25	RO	B	bY		OBr
31	59		Y	Y	Y	OBr
32	45		B	B		OBr
33	62		V	B		Pi
34	56		B	B		O
35	89		Y	Y		Br
36	58		Pi	Y	Y	Pi
37	81		V	B		OBr
38†	59		B	B		L
39†	57		P			Y
40†	92		B	B		PBr
41†	75	Y	P	B	Y	RP
42†	75		B	B	Y	WP
43†	93		B	B		Br
44†	76		V	V		YBr
45†	57					P
46†	91		dB	B	Y	P

*Legend:						
	B	blue	Br	brown	C	cochineal red
	G	green	L	lilac	M	maroon
	O	orange	P	purple	Pi	pink
	R	red	V	violet	W	white
	Y	yellow	b	bright	d	dark

†Known compounds				
	38	aesculin	43	ferulic acid
	39	arbutin	44	gallic acid
	40	caffeic acid	45	hesperidin
	41	catechin	46	simaric acid
	42	chlorogenic acid		

However, when rather distantly related species are compared, it is often more desirable to express the I.V. with respect to the total number of compounds detected in that particular species (I.V.i). Both forms of isolation value were calculated.

RESULTS

Thirty-seven compounds were observed in this study of twelve species. Figure 4 shows the total complement of spots and the R_f values and colour reactions of the individual spots are given in Table 2. The distribution of these compounds in the different species is shown in Table 3. From this table, P.A. Indices were calculated for all possible pairs of the twelve species and the polygonal graphs were drawn (Fig. 5). It is impractical to compare a large number of items in the form of polygonal graphs as a large number of axes would be required, which would render interpretations extremely difficult. Therefore, in species where both leaves and stems are present, the P.A. Indices of the leaves, rather than the stems, were used in drawing the graphs. However, in the case of *Viscum rotundifolium*, where specimens were taken from different host species, four polygonal graphs were drawn. In this way, a comparison could be made between phenolics of stems and leaves and one could also ascertain the influence of the host on the parasites. The isolation values for each species were calculated and recorded in Table 4.

DISCUSSION

Relatively few phenolic compounds were separated out, approximately ten spots per species, when compared with reports in the literature. Ellison, Alston, and Turner (1962) separated between ten and eighteen compounds per species, an average of 13, in a study of the leaves of 17 species of *Bahia*. It appears that *Viscum* and *Loranthus* may be lacking in phenolics. A much wider range of species would, however, have to be considered before reliable generalizations can be made.

On the basis of R_f values and colour reactions, one spot was identified as chlorogenic acid and another was identified as catechin. The former was present in all species of *Viscum*, whereas the latter was found in all species of *Loranthus*.

From the results of the P.A. Indices and polygonal graphs (Fig. 5) a number of interesting facts emerged. In general the species belonging to the genus *Viscum* showed markedly higher affinities with one another than with the species of *Loranthus*. Similarly the species of *Loranthus* showed higher affinities with one another than with species of *Viscum*. On the basis of phenolic composition, therefore, the genera are easily distinguished.

Table 3. Enumeration of the spots (phenolic compounds) present in the stems and/or leaves of each of the species studied.

SPOTS

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
1. *Viscum rotundifolium* leaves (Boscia)	+	+	+	+	+	+	+	+	+	+	+											+						+					+				+
2. *V. rotundifolium* stems (Boscia)	+		+	+	+	+	+	+	+	+	+											+						+					+				
3. *V. rotundifolium* leaves (Ziziphus)	+	+	+	+	+		+	+	+	+	+											+				+		+					+				
4. *V. rotundifolium* stems (Ziziphus)	+		+	+	+	+	+	+	+	+	+											+						+									
5. *V. verrucosum*								+	+	+							+		+			+						+									+
6. *V. capense*								+	+	+							+		+							+		+			+	+					+
7. *V. obovatum* leaves									+	+		+	+	+		+							+			+		+	+			+		+			+
8. *V. obovatum* stems									+	+		+	+	+	+	+							+			+		+	+		+	+		+			+
9. *V. obscurum* leaves			+	+				+	+	+			+				+			+		+				+			+		+						+
10. *V. obscurum* stems			+	+				+	+	+			+		+		+			+		+				+		+			+	+					+
11. *V. combreticola*							+		+	+		+	+	+	+											+	+	+	+	+		+		+			
12. *Loranthus dregei* leaves										+					+			+			+	+		+				+						+		+	
13. *L. dregei* stems										+					+			+			+	+		+	+			+						+	+	+	
14. *L. rubromarginatus* leaves										+									+	+				+	+	+	+		+						+		
15. *L. rubromarginatus* stems										+									+	+					+		+		+								+
16. *L. zeyheri* leaves										+										+			+	+	+	+	+	+							+		
17. *L. zeyheri* stems										+										+			+	+	+	+	+				+						
18. *L.* sp. cf. *oleaefolius* leaves										+														+	+	+		+			+				+		
19. *L.* sp. cf. *oleaefolius* stems										+										+				+		+					+						
20. *L. oleaefolius* leaves										+			+							+			+	+	+	+	+								+		
21. *L. oleaefolius* stems										+			+							+			+	+	+	+	+	+				+	+				
22. *L. minor* leaves										+			+							+			+	+	+	+											
23. *L. minor* stems										+										+			+	+	+	+											

From the polygonal graphs (Fig. 5) of the *Loranthus* species (Numbers 12–22) it will be seen that they are not very similar to those species of *Viscum* (Numbers 1–11). Note also that *L. rubromarginatus* (14), *L. zeyheri* (16), *L. oleaefolius* (20) and *L. minor* (22) have similar patterns. They therefore seem to form a distinct group differing from the remaining two species. *Loranthus dregei* (12) seems to bear very little resemblance to the other species of the genus, whereas the unknown species (18) does show a pattern resembling, to some extent, that of the 4 similar species mentioned above. This unknown species was later given a dubious identification (at the National Herbarium, Pretoria) as *L. oleaefolius* but it differs in phenolic composition from that species (no. 20).

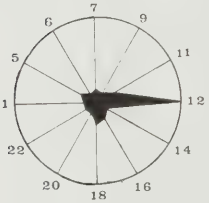
In the case of *Viscum*, 9 polygonal graphs (Fig. 5) were drawn so that a comparison could be shown between the phenolics of leaves and stems in one species, and also the effect of different hosts. The first two graphs depict the phenolic composition of *V. rotundifolium* growing on *Boscia albitrunca*. The next two are also of this parasite but growing on the host *Ziziphus mucronata*. The pattern of the graphs is similar but there is greater similarity between the phenolics of the stems of the parasite growing on the same host. In other words, the effect of different hosts on the phenolic content of the parasites is of less importance in this case than the part of the plant used. There was a P.A. Index of 81,3% between the leaves growing on different hosts and one of 92,3% between the stems, thus indicating that the leaves are affected by the host on which they grow to a greater degree than the stems. These affinity values are high and the differences due to the different hosts insignificant when the generally very low affinities between the other species are considered. *Ziziphus* and *Boscia* may have similar phenolics, thus accounting for the similarities observed in the parasites, but they are only distantly related, belonging to the Rhamnaceae and Capparidaceae respectively, and this explanation seems unlikely. It is more likely, in this case, that the phenolic composition of the host, although having a slight influence, is not so important as to dominate the phenolics of the parasites growing on it. However, in view of the very complex interrelationships of host and parasite, no generalization can validly be made about these translocation relationships without a more thorough investigation.

On examining the polygonal graphs (Fig. 5) of the other species some similarities between *Viscum* species emerge. *Viscum capense* (6) and *V. verrucosum* (5) have fairly similar patterns. Morphologically, they appear very similar, both having rounded cladodes. However, the third species without leaves, *V. combreticola* (11), did not show a high affinity with either of these species, but rather had similarities to *V. obovatum* (7). *Viscum combreticola* differs markedly from the other leafless species, possessing cladodes that are broad

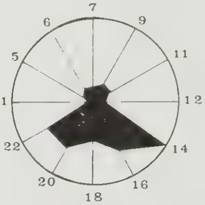
TABLE 4.

Isolation values for species of Viscum and Loranthus.

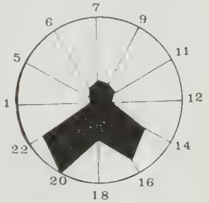
Taxon	*Number of unique compounds*	*Total compounds*	*I.V.n.*	*I.V.i.*
1. *V. rotundifolium* leaves (*Boscia*)	5	15	13,5	33,3
2. *V. rotundifolium* stems (*Boscia*)	5	13	13,5	38,5
3. *V. rotundifolium* leaves (*Ziziphus*)	5	14	13,5	35,7
4. *V. rotundifolium* stems (*Ziziphus*)	5	12	13,5	41,7
5. *V. verrucosum*	0	8	0	0
6. *V. capense*	0	10	0	0
7. *V. obovatum* leaves	1	13	2,7	7,7
8. *V. obovatum* stems	1	15	2,7	6,7
9. *V. obscurum* leaves	0	13	0	0
10. *V. obscurum* stems	0	15	0	0
11. *V. combreticola*	1	14	2,7	7,1
12. *L. dregei* leaves	3	9	8,1	33,3
13. *L. dregei* stems	3	11	8,1	27,3
14. *L. rubromarginatus* leaves	0	9	0	0
15. *L. rubromarginatus* stems	0	7	0	0
16. *L. zeyheri* leaves	0	9	0	0
17. *L. zeyheri* stems	0	8	0	0
18. *L.* sp. leaves	0	7	0	0
19. *L.* sp. stems	0	5	0	0
20. *L. oleaefolius* leaves	0	9	0	0
21. *L. oleaefolius* stems	0	11	0	0
22. *L. minor* leaves	0	7	0	0
23. *L. minor* stems	0	6	0	0



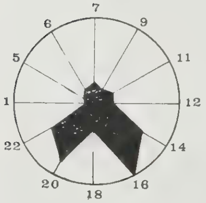
L. dregei



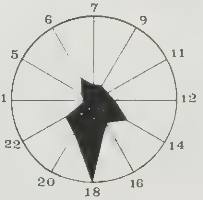
L. rubromarginatus



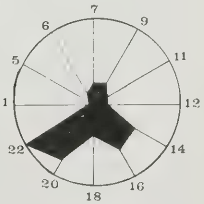
L. oleaefolius



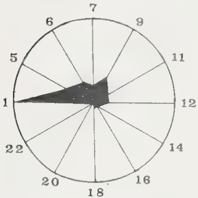
L. zeyheri



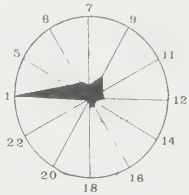
L. species



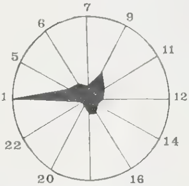
L. minor



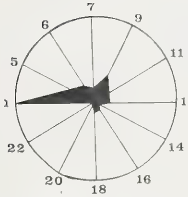
V.rotundifolium (leaves)
on Boscia albitrunca



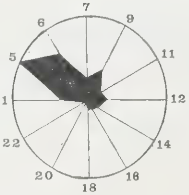
V.rotundifolium (stems)
on B.albitrunca



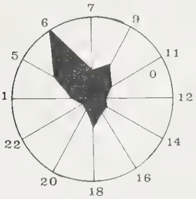
V. rotundifolium (leaves)
on Ziziphus mucronata



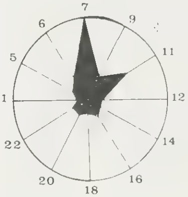
V.rotundifolium (stems)
on Z. mucronata



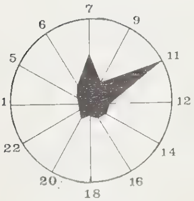
V. verrucosum



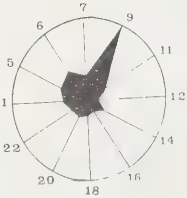
V.capense



V.obovatum



V.combreticola



V.obscurum

FIG. 5.
Polygonal graphs constructed on the basis of P.A. indices for each of the 12 species.

and flattened. *Viscum obscurum* (9) is not very much like any of the other species, probably being most like *V. obovatum.*

The isolation values (Table 4) emphasize the distinctiveness of *V. rotundifolium* and *L. dregei* from the other species. Similarly, to a lesser degree, *V. obovatum* and *V. combreticola* are seen to differ. It should be noted that half the species of *Viscum* possess at least one unique compound as opposed to a single species of *Loranthus* with unique compounds. This again seems to indicate that the species of *Loranthus* form a more homogeneous group than those of *Viscum*. Because of the general lack of unique compounds (67% had none), the different species cannot be differentiated on this basis.

General Discussion and Conclusions

The narrowness of the spectrum of species investigated obviously limits the interpretation of the results. By studying a much wider range of species of each genus, it should be possible to draw conclusions of general applicability. Such studies may well indicate whether certain species of *Viscum* can in fact be distinguished by unique compounds and whether species of *Loranthus* generally have different combinations of the same phenolics rather than unique or characteristic compounds. It should also be possible to determine whether or not further species could be placed with the four similar *Loranthus* species found in this study to form a distinctive group, and whether others could be grouped with *V. capense* and *V. verrucosum* or with *V. combreticola* and *V. obovatum.*

Loranthus dregei, which was shown to be different from the other species of *Loranthus* studied, has been placed in a separate genus *Erianthemum*, whereas the other species are considered to be species of *Tapinanthus* by some workers (Danser, 1933; Balle, 1956, 1968). This study would support the separation of *Loranthus* with respect to these species, although our conclusions are somewhat limited, as so few species have been studied for phenolics. Similarly any conclusions reached about the sectional classification of *Viscum* are limited by the narrow range of species studied.

With respect to the subdivision of the family into two separate families, evidence from the phenolic compounds would support this view. The chromatographic data clearly indicated definite differences between the phenolic compounds of *Viscum* and *Loranthus* species, but it is difficult to decide whether these differences would indicate family differences rather than subfamily or even only generic differences. It seems more acceptable to take the view of Barlow (1964) and Kuijt (1968) that there are two separate families and that the phenolic compounds give further evidence for this subdivision.

In addition, biochemical studies of the phenolics and other compounds may provide some clues as to the host specificity, which appears to vary widely from relatively unspecific forms such as *V. rotundifolium* and *L. dregei* to more highly specific forms such as *V. verrucosum* and *V. combreticola*. Thorough investigations of host-parasite relationships are essential. These interrelationships are a critical factor in deciding whether studies of phenolic constituents will be of general use in gaining a better understanding of the taxonomy of the family or whether, in fact, the relationships are too variable and complex depending ultimately on the nature of the host. If the latter is true chemotaxonomic studies may well be too tedious and time-consuming to be of practical value. However, in this study on limited investigation of the variability of the phenolics of *Viscum rotundifolium* growing on different hosts, there was very little variability encountered. Whether these results can be extrapolated to other species is not known.

The chemical characters studied here show chemical relationships between the Loranthaceae species, but they are just one aspect in a complex mosaic. These characters have been shown to be useful and valid but it seems that chemical characters can, at least at present, best be used in conjunction with as many other sources of evidence as possible to obtain a classification that is both natural and phylogenetic.

Acknowledgments

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